1. (Amended) A method for selectively inducing production of butyrate by bacteria in the human colon comprising administering D-tagatose to a human in an amount effective to induce production of butyrate.

7. (Amended) A method for selectively stimulating growth of lactobacilli and lactic acid bacteria in the human colon comprising administering D-tagatose to a human in an amount effective to stimulate growth of lactobacilli and lactic bacteria in the human colon.

REMARKS

Claims 1-12 remain pending in this application. Claims 1 and 7 have been amended to more clearly define the subject matter which applicants regard as their invention. The use of D-tagatose produces the selective production of butyrate and the selective stimulation of growth of lactobacilli and lactic bacteria in the human colon as described in the specification, for example, in the sentence bridging pages 6 and 7; page 13, lines 24-34; page 15, lines 15-17; and page 18, lines 2-13. Accordingly, no new matter has been introduced by this amendment.

The objections raised by the Official Draftsperson to the drawings in this application have been noted. We request that any requirement to correct the drawings be held in abeyance until such time as the Examiner has determined that the claims in this application are patentable.

The first paragraph of the specification has been amended to make reference to an earlier filed provisional application in accordance with 37 C.F.R. § 1.78(a)(4).

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Contrary to the indication in the Office Action, this application was not filed under the provisions of 37 C.F.R. § 1.60, which was abolished as of December 1, 1997. The instant application was filed February 23, 1999. Further, contrary to the statement by the Examiner, there is no need to identify the relationship (continuation or continuation-in-part) between the non-provisional and provisional applications. See MPEP 201.11 [Reference to first application].

Claims 1-12 have been rejected under 35 U.S.C. § 103 as being unpatentable over Zehner (U.S. Patent No. 4,786,722) in view of the combination of Morelli et al. (U.S. Patent No. 5,709,857), MacFarlane et al. (The Large Intestine:Physiology, Pathophysiology and Disease (1991)) and Mortensen et al. (American Institute of Nutrition (1987)). The Examiner has argued that Mortensen et al. and MacFarlane et al. teach the production of butyrate from monosaccharide and disaccharide substrates broadly in the human colon, which differs from the claimed invention only with respect to the fact that D-tagatose is not specifically cited. The Examiner further notes that Zehner teaches that unabsorbable sugars such as D-tagatose are subject to fermentation in the human colon, and that Morelli teaches that several species of Lactobacillus positively ferment carbohydrates such as Tagatose.

The Examiner concludes that it would have been *prima facie* obvious to use D-tagatose or any other saccharide which may be positively fermented by indigenous microflora of the human large intestine in a method for inducing production of butyrate or stimulating the growth of Lactobacilli and lactic acid bacteria. A person of ordinary skill in the art would have been motivated, according to the Examiner, to use D-

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tagatose because it is not broken down until it traverses into the large intestine, enabling the growth of positive flora such as Lactobacilli and also allowing for production of short chain fatty acids (SCFAs), specifically butyrate. Further according to the Examiner, one of skill in this art would have a reasonable expectation of success with the production of butyrate or the growth of Lactobacilli with not only D-tagatose, but any other mono or disaccharide which escapes digestion in the small intestine and has been indicated by the prior art as a substrate for commensalistic flora from which SCFAs such as butyrate are produced.

This rejection is respectfully traversed.

The present invention is directed to the recognition by the inventors that D-tagatose induces selective production of butyrate in the colon and selectively stimulates the growth of beneficial Lactobacilli and lactic acid bacteria in the human colon at levels that are not contemplated or expected from the teachings of the prior art. A prerequisite for a carbohydrate or a fiber to be prebiotic candidate is that it has to reach the colon and be a substrate for the beneficial bacteria of concern. Those skilled in this art recognize that a prebiotic component is one that selectively promotes the growth and/or the activity of one of a limited number of bacteria in the colon. See Roberfrold et al., "*Colonic Microfilora: Nutrition and Health", Nutrition Reviews*, Vol. 53, No. 6, pp. 127-130 at p. 129 (May 1995). As recognized by the Examiner, Morelli et al. '857 shows that Lactobacillus species isolated from human colon are able to degrade a large variety of carbohydrates including well known and commercial malabsorbed carbohydrates like mannitol and maltose. This observation, however, does not mean

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that these two carbohydrates selectively promote growth of Lactobacilli in the competitive environment of the colon. Growth on various sugars is an old and well recognized tool to characterize bacteria and discriminate between genera and species. These published data, however, do not give any idea on how well bacteria grow on these substrates.

In order for a carbohydrate to promote growth of Lactobacilli in the colon, it has to be selectively degraded by Lactobacilli in the highly competitive environment of the colon. Most other bacteria and numerous colon bacteria have, similar to Lactobacillus, a rather broad ability to degrade malabsorbed carbohydrates like fibers, polyols and sugars, and thus prevent selective growth of beneficial bacteria like Lactobacillus by simple competition. The surprising and unexpected characteristics of D-tagatose are that only very few human intestinal bacteria are able to degrade D-tagatose. This high selectivity of D-tagatose among intestinal bacteria, and the widespread ability and very efficient utilization among Lactobacillus, appears to be the explanation for the D-tagatose induced enrichment of colon Lactobacillus.

SCFAs like acetate, propionate and butyrate are normal end products of colonic fermentation of malabsorbed or undigested carbohydrates and are found in fairly constant proportion in the human colon, with acetate as the dominant SCFA. As noted in Mortensen et al., cited by the Examiner, it is speculated that saccharide fermentation always results in formation of acetate, and the relative production of acetate, propionate and butyrate is related to the monosaccharide composition of dietary fiber available for colonic bacteria. (See abstract). As noted at column 2, page 321, the biochemical

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mechanisms of these *in vitro* observations are difficult to access *in vivo*. Further, MacFarlane et al. teaches (page 6, column 1) that intestinal bacteria degrade different polysaccharides at different rates, and that (page 63, column 2) one problem with studies of SCFA production and metabolism is that each fatty acid is metabolized differently and at a number of sites in the body. Carbohydrate fermentation in the human colon is difficult to study *in vivo*, and the comparison of the results of *in vitro* experiments with the *in vivo* human data was described as difficult at col. 2, p. 216 of Edwards et al. (*In Vitro Method for Quantification of the Fermentation of Starch by Human Faecal Bacteria*, J. Sci. Food Agric. 71:209-217 (1996).

Accordingly, while it may be obvious that an increased ingestion of malabsorbed or undigested carbohydrate gives a general stimulation of colon fermentation, and thus a general stimulation of production of SCFAs including butyrate, these observations do not predict the unique ability of D-tagatose to selectively induce production of butyrate and selectively stimulate growth of Lactobacilli and lactic bacteria in the human colon as recited in the claims of the present application. Specifically, the stimulation of SCFA production by D-tagatose gives a specific increase of butyrate as can be seen in the increased mol percent of butyrate in Tables 1 and 2 of the present specification. As can further be observed from Tables 1 and 2, butyrate is produced at the expense of acetate. There is nothing in the prior art relied upon by the Examiner that would permit those of ordinary skill of this art to predict this unique capability of D-tagatose.

Enclosed is a figure showing acetate concentrations in pig intestine after intake of increasing amounts of D-tagatose. This figure even shows a decline in the absolute

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concentrations of acetate. The data are drawn from the same experiment yielding the intestinal butyrate concentrations in Fig. 1 (example 2). The control pigs were fed a normal pig basal diet containing fermentable fiber + 20% sucrose and in the tagatose groups, tagatose was added in lieu of sucrose. The production of SCFA in the control pigs was a result of fermentation of basal diet fiber and in the tagatose groups a combination of basal diet fiber and malabsorbed D-tagatose. The decrease in absolute intestinal acetate concentration means that not only was D-tagatose preferentially fermented into butyrate, but also that the D-tagatose influence on intestinal flora also affected the normal fermentation of fibers into acetate towards production of butyrate. Thus, the D-tagatose induced production of butyrate is not just a general stimulation of fermentation, but rather a selective stimulation of butyrate at the expense of acetate. The D-tagatose stimulation of colonic butyrate production has been confirmed in both *in vitro* and *in vivo* studies (Examples 1-5).

The *in vitro* stimulation of butyrate by sorbitol, D-galacturonic acid and D-glucuronic acid reported by Mortensen et al. (1988), has not been confirmed in any *in vivo* studies to the knowledge of the inventors. Sorbitol and sorbitol containing polyols like maltitol, lactitol, isomalt and HSH (hydrogenated starch hydrolysate) have been commercial for more than 10 years and no literature, to the knowledge of the inventors, has ever claimed an *in vivo* stimulation of colonic butyrate production. A study setup in pigs, similar to the *in vivo* absorption of SCFA described in Example 3 was performed with maltitol syrup (Shyd). *See* Rerat et al., "VFA Absorption After Maltitol Intake in Pigs," pp. 2473-2488 (1996). Pigs had 800 g of a diet containing 53% SHyd of which

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44% is sorbitol (187 g sorbitol) and large intestinal absorption of SCFA was measured. Most of maltitol is hydrolysed to glucose and sorbitol in the small intestine, which means that the delivered substrate to fermentation in the large intestine is mainly sorbitol. *See* Rerat, A., "Influence of carbohydrates on reducing sugars and VFA in Pigs," pp. 3-19 (1996). Rerat (1996) concludes on page 8 and table II, that "sorbitol residues (132g) in the gut produced large amounts of propionic acid." The conclusion from the Rerat *in vivo* study is that sorbitol stimulates propionic acid and not butyric acid. Copies of the Rerat articles are attached for the Examiner's convenience.

The potential beneficial effect of butyrate on colonic epithelial cells has been known for the last 10 to 20 years. Nevertheless, there has been a continued search for undigestable and malabsorbed carbohydrates which specifically induce production of butyrate. Other attempts to solve this problem have identified resistant starch as a candidate that has shown a slight increase in butyrate mol percent in some studies, but nothing that would suggest to a person of ordinary skill in the art that D-tagatose possesses this unique capability. *See*, for example, Asp et al., "Nutritional Implications of Resistant Starch," Nutrition Research Reviews, 1-31 (1996); Berggren et al., "Short-Chain Fatty Acid Content and pH in Caecum of Rats Fed Various Sources of Starch," J. Sci. Food Agric. 68:241-248 (1995); and Martin et al. "Production of Short-Chain Fatty Acids from Resistant Starch in a Pig Model," J. Sci. Food Agric. 77:71-80 (1998). Copies of these documents are attached for the Examiner's convenience.

It is respectfully submitted that the prior art does not provide any motivation or suggestion to use D-tagatose as a monosaccharide that would selectively induce

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production of butyrate or selectively provide for the stimulated growth of Lactobacilli and lactic bacteria in the human colon as recited in the claims in this application. Further, there is no information in the prior art relied upon in this application that would permit a person of ordinary skill in the art to predict that there would be a selective inducement and stimulation based on the use of D-tagatose. As the prior art relied upon by the Examiner fails to establish a *prima facie* case of obviousness, it is respectfully requested that this ground of rejection be withdrawn.

Reexamination and reconsideration of this application in view of the amendments and remarks provided above is respectfully requested.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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By: Charles E Van Horn
Charles E. Van Horn
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Dated: March 1, 2000
Enclosures: Figure
Roberfrold et al. article
Edwards et al. article
Two Rerat articles
Asp et al. article
Berggren et al. article
Martin et al. article

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